

THAT WHICH IS CLAIMED:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - 5 (a) the nucleotide sequence shown in SEQ ID NO:1;
 - (b) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2;
 - (c) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the nucleotide sequence shown in SEQ ID NO:1 under stringent conditions;
 - 10 (d) the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;
 - (e) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021 under stringent conditions;
 - 15 (f) a nucleotide sequence encoding an MLH1 polypeptide, said sequence having at least about 75% sequence identity to the nucleotide sequence shown in SEQ ID NO:1;
 - (g) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;
 - 20 (h) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide sequence shown in SEQ ID NO:2; and
 - 25 (i) a nucleotide sequence comprising an antisense sequence corresponding to the nucleotide sequence in (a), (b), (c), (d), (e), (f), (g), or (h).
2. An expression cassette comprising a nucleic acid molecule of claim 1, wherein said nucleotide sequence is operably linked to a promoter that drives expression in a plant cell.

3. The expression cassette of claim 2, wherein said promoter is selected from the group consisting of constitutive, chemically regulatable, and tissue-preferred promoters.

5

4. An isolated nucleic acid molecule comprising a fragment of SEQ ID NO:1, said fragment comprising at least 27 contiguous nucleotides of a nucleotide sequence selected from the group consisting of:

- 10 and
- (a) nucleotides 1-2283 of the nucleotide sequence of SEQ ID NO:1;
- (b) nucleotides 1-2283 of the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021.

15 5. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence comprising at least 60 nucleotides that encodes a fragment of the amino acid sequence set forth in SEQ ID NO:2; and
- (b) a nucleotide sequence comprising at least 60 nucleotides that encodes a fragment of the amino acid sequence encoded by the cDNA insert of the 20 plasmid deposited with ATCC as Patent Deposit No. PTA-2021.

6. A host cell engineered to express any one of the nucleic acid molecules of claims 1, 4, or 5.

25 7. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence set forth in SEQ ID NO:2;
- (b) an amino acid sequence having at least 75% sequence identity to the amino acid sequence set forth in SEQ ID NO:2; and

(c) an amino acid sequence comprising at least 20 consecutive amino acids of the amino acid sequence set forth in (a) or (b).

8. A genetically modified rice plant comprising in its genome an endogenous 5 *MLH1* gene having a mutation within said gene, wherein said endogenous *MLH1* gene corresponds to the cDNA set forth in SEQ ID NO:1 and said mutation is due to the presence of a transposon.

9. Genetically modified seed of said plant of claim 8.

10 10. A transformed plant comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a chemical-inducible promoter that drives expression in said plant cell, wherein said nucleotide sequence comprises a nucleotide sequence selected from the group consisting 15 of:

(a) the nucleotide sequence shown in SEQ ID NO:1;
(b) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2;

20 (c) a nucleotide sequence encoding an *MLH1* polypeptide, wherein said nucleotide sequence hybridizes to the nucleotide sequence shown in SEQ ID NO:1 under stringent conditions;
(d) the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;

25 (e) a nucleotide sequence encoding an *MLH1* polypeptide, wherein said nucleotide sequence hybridizes to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021 under stringent conditions;

(f) a nucleotide sequence encoding an *MLH1* polypeptide, said sequence having at least about 75% sequence identity to the nucleotide sequence shown in SEQ ID NO:1;

- (g) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;
- (h) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide sequence shown in SEQ ID NO:2;
- 5 (i) a nucleotide sequence consisting of at least 27 consecutive nucleotides of nucleotides 1-2238 of (a) or (d); and
- (j) a nucleotide sequence comprising an antisense sequence corresponding to the nucleotide sequence in (a), (b), (c), (d), (e), (f), (g), (h), or (i).

10

11. A transformed plant comprising in its genome:

- (a) a first stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said first expression cassette is located between two FRT sequences oriented to allow for inversion or excision of said first expression cassette by FLP recombinase, said nucleotide sequence selected from the group consisting of:
- (i) the nucleotide sequence shown in SEQ ID NO:1;
- (ii) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2;
- 15 (iii) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the nucleotide sequence shown in SEQ ID NO:1 under stringent conditions;
- (iv) the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;
- 20 (v) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021 under stringent conditions;
- (vi) a nucleotide sequence encoding an MLH1 polypeptide, said sequence having at least about 75% sequence identity to the nucleotide sequence shown
- 25 in SEQ ID NO:1;

30

(vii) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;

5 (viii) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide sequence shown in SEQ ID NO:2;

(ix) a nucleotide sequence consisting of at least 27 consecutive nucleotides of nucleotides 1-2238 of (a) or (d); and

10 (x) a nucleotide sequence comprising an antisense sequence corresponding to the nucleotide sequence in (i), (ii), (iii), (iv), (v), (vi), (vii), (viii) or (ix); and

(b) a second stably incorporated expression cassette comprising a nucleotide sequence encoding said FLP recombinase operably linked to a chemical-inducible promoter that drives expression in said plant.

15

12. A transformed plant comprising in its genome at least one stably incorporated expression cassette, wherein said expression cassette comprises a nucleotide sequence operably linked to a heterologous chemical-inducible promoter that drives expression in said plant cell, wherein said nucleotide sequence encodes a mutated MLH1 polypeptide with defective mismatch repair activity due to mutagenesis of at least one amino acid residue necessary for normal mismatch repair activity, wherein said mutated MLH1 polypeptide binds substrate with an affinity similar to that observed for a corresponding non-mutated endogenous MLH1 enzyme.

25

13. A transformed plant comprising in its genome:

(a) a first stably incorporated expression cassette comprising a lexA DNA binding site embedded in a tissue-specific promoter that drives expression in a plant cell, wherein said tissue-specific promoter is operably linked to a first nucleotide sequence comprising a nucleotide sequence selected from the group consisting of:

30

(i) the nucleotide sequence shown in SEQ ID NO:1;

- (ii) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2;
- (iii) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the nucleotide sequence shown in SEQ ID NO:1 under stringent conditions;
- (iv) the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;
- (v) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021 under stringent conditions;
- (vi) a nucleotide sequence encoding an MLH1 polypeptide, said sequence having at least about 75% sequence identity to the nucleotide sequence shown in SEQ ID NO:1;
- (vii) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;
- (viii) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide sequence shown in SEQ ID NO:2;
- (ix) a nucleotide sequence consisting of at least 27 consecutive nucleotides of nucleotides 1-2238 of (a) or (d); and
- (x) a nucleotide sequence comprising an antisense sequence corresponding to the nucleotide sequence in (i), (ii), (iii), (iv), (v), (vi), (vii), (viii) or (ix); and
- (b) a second stably incorporated expression cassette comprising of a second nucleotide sequence encoding a lexA repressor operably linked to a chemical-inducible promoter that drives expression in a plant cell.

14. Transformed seed of the plant of any one of claims 10, 11, 12, or 13..

15. The transformed plant of any one of claims 10, 11, 12, or 13, wherein said plant is a monocot.

16. The transformed plant of claim 15, wherein said monocot is rice, maize,
5 wheat, barley, sorghum, or rye.

17. A method for increasing the efficiency of targeted gene mutation or homologous recombination in a plant, said method comprising:

- (a) transposon tagging an endogenous *MLH1* gene in said plant;
- 10 (b) transforming said plant with nucleic acid comprising a nucleotide sequence having at least one desired mutation or at least one nucleotide sequence to be homologously recombined; and
- (c) selecting said transformed plants that contain said mutation or said homologously recombined nucleotide sequence.

15

18. The method of claim 17, wherein said plant is rice and wherein said *MLH1* gene corresponds to the nucleotide sequence set forth in SEQ ID NO:1.

19. A method for increasing the efficiency of targeted gene mutation or
20 homologous recombination in a plant, said method comprising:

- (a) transforming said plant with at least one expression cassette comprising a nucleotide sequence operably linked to a chemical-inducible promoter that drives expression in a plant cell, wherein said nucleotide sequence comprises a nucleotide sequence selected from the group consisting of:
 - (i) the nucleotide sequence shown in SEQ ID NO:1;
 - (ii) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2;
 - (iii) a nucleotide sequence encoding an *MLH1* polypeptide, wherein said nucleotide sequence hybridizes to the nucleotide sequence shown in SEQ ID NO:1 under stringent conditions;

(iv) the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;

5 (v) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021 under stringent conditions;

(vi) a nucleotide sequence encoding an MLH1 polypeptide, said sequence having at least about 75% sequence identity to the nucleotide sequence shown in SEQ ID NO:1;

10 (vii) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;

(viii) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide sequence shown in SEQ ID NO:2;

15 (ix) a nucleotide sequence consisting of at least 27 consecutive nucleotides of nucleotides 1-2238 of (a) or (d); and

(x) a nucleotide sequence comprising an antisense sequence corresponding to the nucleotide sequence in (i), (ii), (iii), (iv), (v), (vi), (vii), (viii) or (ix);

20 (b) transforming said plant with nucleic acid comprising a nucleotide sequence having at least one desired mutation or at least one nucleotide sequence to be homologously recombined, wherein said transforming occurs in the presence of a chemical compound capable of inducing said chemical-inducible promoter, whereby said plant's cellular mismatch repair system is inhibited; and

25 (c) selecting said transformed plants that contain said mutation or said homologously recombined nucleotide sequence.

20. A method for increasing the efficiency of targeted gene mutation or homologous recombination in a plant, said method comprising:

30 (a) transforming said plant with a first expression cassette comprising a nucleotide sequence operably linked to a first chemical-inducible promoter that drives

expression in a plant cell, wherein said first expression cassette is located between two FRT sequences oriented to allow for inversion or excision of said first expression cassette by FLP recombinase; wherein said nucleotide sequence is selected from the group consisting of:

- (b) transforming said plant with a second expression cassette comprising a nucleotide sequence encoding said FLP recombinase operably linked to a second chemical-inducible promoter that drives expression in said plant;
- (c) transforming said plant with nucleic acid comprising a nucleotide sequence having at least one desired mutation or at least one nucleotide sequence to be homologously recombined in the presence of a chemical compound capable of inducing expression by said first chemical-inducible promoter, whereby said plant's cellular mismatch repair system is inhibited;
- (d) contacting said plant with a chemical compound capable of inducing expression of said second chemical-inducible promoter thereby inducing expression of FLP recombinase to release said inhibition of the cellular mismatch repair system; and
- (e) selecting said transformed plants containing said mutation or said homologously recombined nucleotide sequence.
- 15
21. A method for increasing the efficiency of targeted gene mutation or homologous recombination in a plant, said method comprising:
- (a) transforming said plant with nucleic acid comprising a nucleotide sequence having at least one desired mutation or at least one sequence to be homologously recombined in the presence of an antibody that selectively binds to and inhibits mismatch repair activity of a polypeptide comprising an amino acid sequence selected from the group consisting of:
- (i) the amino acid sequence set forth in SEQ ID NO:2;
- (ii) an amino acid sequence comprising at least 75% sequence identity to the amino acid sequence set forth in SEQ ID NO:2; and
- (iii) an amino acid sequence comprising at least 20 consecutive amino acids of the amino acid sequence set forth in (i) or (ii); and
- (b) selecting said plants containing said mutation or said homologously recombined nucleotide sequence.
- 25

22. A method for increasing the efficiency of targeted gene mutation or homologous recombination in a plant, said method comprising:

(a) transforming said plant with at least one expression cassette comprising a nucleotide sequence operably linked to a heterologous chemical-inducible promoter that drives expression in said plant cell, wherein said nucleotide sequence encodes a mutated MLH1 polypeptide with defective mismatch repair activity due to mutagenesis of at least one amino acid residue necessary for normal mismatch repair activity, wherein said mutated MLH1 polypeptide binds substrate with an affinity similar to that observed for a corresponding non-mutated endogenous MLH1 enzyme;

10 (b) transforming said plant with nucleic acid comprising a nucleotide sequence having at least one desired mutation or at least one sequence to be homologously recombined, wherein said transforming occurs in the presence of a chemical compound capable of inducing said chemical-inducible promoter, thereby inducing expression of said MLH1 polypeptide with defective mismatch repair activity;

15 and

(c) selecting said plants that contain said mutation or said homologously recombined nucleotide sequence.

23. The method of any one of claims 17, 19, 20, 21, or 22, wherein said nucleic acid comprising the nucleotide sequence having the desired mutation or the nucleotide sequence to be homologously recombined is that of a species different from said plant being transformed, whereby a hybrid plant species is formed.

24. A method for detecting, locating, or removing at least one base pair mismatch in a double-stranded nucleic acid molecule, said method comprising:

(a) providing a nucleic acid duplex comprising at least one base pair mismatch;

(b) contacting said nucleic acid duplex with an isolated polypeptide possessing MLH1 mismatch recognition activity, either alone or in combination with

other mismatch repair proteins, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of:

- (i) the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) an amino acid sequence having at least 75% sequence identity to the amino acid sequence set forth in SEQ ID NO:2; and
 - (iii) an amino acid sequence comprising at least 20 consecutive amino acids of the amino acid sequence set forth in (i) or (ii); and
- (c) detecting any complex between said nucleic acid duplex and said polypeptide as a measure of the presence of said base pair mismatch in the nucleic acid duplex.

25. The method of claim 24, wherein the detection or removal of said complex comprises the use of an antibody that binds selectively to said polypeptide.

15 26. The method of claim 24, wherein said base pair mismatch is a SNP.

27. A method for producing reversible male sterility in a plant, said method comprising:

- (a) transforming a plant with a first expression cassette comprising of a lexA DNA binding site embedded in a tissue-specific promoter that drives expression in said plant operably linked to a first nucleotide sequence that when expressed disrupts pollen formation or function through inhibition of said plant's cellular mismatch repair system, wherein said first nucleotide sequence is selected from the group consisting of:
 - (i) the nucleotide sequence shown in SEQ ID NO:1;
 - (ii) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2;
 - (iii) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the nucleotide sequence shown in SEQ ID NO:1 under stringent conditions;

- (iv) the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;
- (v) a nucleotide sequence encoding an MLH1 polypeptide, wherein said nucleotide sequence hybridizes to the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021 under stringent conditions;
- 5 (vi) a nucleotide sequence encoding an MLH1 polypeptide, said sequence having at least about 75% sequence identity to the nucleotide sequence shown in SEQ ID NO:1;
- (vii) a nucleotide sequence encoding an MLH1 polypeptide 10 having at least about 75% sequence identity to the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Patent Deposit No. PTA-2021;
- (viii) a nucleotide sequence encoding an MLH1 polypeptide having at least about 75% sequence identity to the polypeptide sequence shown in SEQ 15 ID NO:2;
- (ix) a nucleotide sequence consisting of at least 27 consecutive nucleotides of nucleotides 1-2238 of (a) or (d); and
- (x) a nucleotide sequence comprising an antisense sequence corresponding to the nucleotide sequence in (i), (ii), (iii), (iv), (v), (vi), (vii), (viii) or (ix);
- (c) transforming said plant with a second expression cassette 20 comprising a second nucleotide sequence encoding a lexA repressor protein operably linked to a chemical-inducible promoter that drives expression in said plant; and
- (d) exposing said plant to a compound capable of inducing said chemical-inducible promoter, thereby inducing expression of said lexA repressor protein, whereby inhibition of the cellular mismatch repair system is released and said male 25 sterility is reversed.

28. The method of claim 27, wherein said tissue-specific promoter is an anther-specific promoter and said chemical-inducible promoter is a herbicidal safener.

29. An antibody that binds selectively to a polypeptide selected from the group consisting of:

- (a) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2;
- 5 (b) a polypeptide comprising at least 75% sequence identity to the amino acid sequence set forth in SEQ ID NO:2; and
- (c) a polypeptide comprising at least 20 consecutive amino acids of the amino acid sequence set forth in (a) or (b).

SEARCHED
INDEXED
SERIALIZED
FILED
APR 12 1999
U.S. PATENT AND TRADEMARK OFFICE